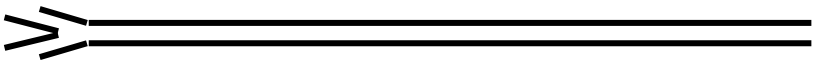
Chapter 4



Method Validation Requirements

4.1 Introduction

Method validation is the process by which a laboratory or vendor establishes the performance of a new method or substantiates the performance of a method modification. New and modified methods must be validated to prove that they accurately measure the concentration of an analyte in an environmental sample. In keeping with the intent of streamlining and flexibility, EPA proposes to establish validation requirements that reflect the level of intended use of the method. This is accomplished through a three-tiered approach, as shown in Table 4-1.

Table 4-1: Application of Method Tiers

Tier Level	Laboratory Use	Applicable to . . .
Tier 1	Single Laboratory	One or more matrix types from any industry; or one or more PWSs
Tier 2	All Laboratories	One or more matrix types within one industrial category or subcategory; or all PWSs
Tier 3	All Laboratories	All matrix types from all industrial categories and subcategories

Under Tier 1, single laboratories will be allowed to validate and use modified methods without the burden of conducting an interlaboratory method validation study. Modified methods intended for multi-laboratory use in a given industrial category or subcategory (Tier 2) or nationwide use (Tier 3) require interlaboratory testing.

All new and modified methods must be validated to demonstrate that the method is capable of yielding reliable data for compliance monitoring purposes under the Clean Water Act or Safe Drinking Water Act. The same tests are performed to validate new and modified methods; however, the results are used differently. Test results from validation of a new method are used to develop quality control (QC) acceptance criteria for that method, whereas test results from validation of a modified method are used to demonstrate that the modified method produces results equivalent or superior to results produced by the reference method.

Method modifications are considered to be approved by EPA and may be used after successful validation and documentation at the appropriate tier. For new methods, the validation study must be submitted to EPA and the new method must be approved by EPA before the method can be used for compliance monitoring. Requirements for submitting validation documentation and seeking method approval are provided in Chapter 5.

Although many compliance monitoring analyses are performed by contract laboratories on behalf of a regulated entity, the responsibility for maintaining validation documentation for new and modified methods rests with the regulated entity. Regulated entities, therefore, must inform their contract laboratories about the requirements for detailed documentation of method modifications that are specified in this chapter.

The key concepts presented and discussed in this chapter are: *method validation, Tiers 1-3, industrial category, industrial subcategory, matrix type, matrix effect, sample matrix effect validation, facility, public water system, sample medium,* and *sample matrix*.

4.2 Summary of Validation Requirements

Requirements for validation depend on the tier to which the new or modified method will be applied. Validation requirements are summarized in Table 4-2. Table 4-2 specifies the numbers of matrix types and facilities or PWSs that must be tested and the numbers and types of analyses required to validate a new or modified method at each tier. To clarify the use of the term “matrix type,” as compared to the terms “sample medium” and “sample matrix,” a **sample medium** is the common name for the physical phase of a sample matrix. Air, water, soil, and sludge are sample media. A **matrix type** is a sample medium with common characteristics across a given industrial category or subcategory. For example, C-stage effluents from chlorine bleach mills, effluent from the continuous casting subcategory of the iron and steel industrial category, POTW sludge, and in-process streams in the Atlantic and Gulf Coast Hand-shucked Oyster Processing subcategory are each a matrix type. For the purposes of this initiative, all drinking waters constitute a single matrix type. A **sample matrix** is the component or substrate that contains the analytes of interest. For purposes of sample collection, "sample matrix" is synonymous with "sample".

As used in Table 4-2, a **facility** is a plant or group of plants within a single location that is regulated under a single National Pollutant Discharge Elimination System (NPDES) permit and/or SDWA. A single facility may have multiple water supplies, discharges, waste streams, or other environmental media that are subject to compliance monitoring. For example, a single facility within the Pulp, Paper, and Paperboard industrial category may have a direct discharge, an indirect discharge, and an in-process waste stream that are all subject to compliance monitoring.

Table 4-2. Summary of Validation Requirements for New Methods and Method Modifications[1]

Method Application	Number of			Number of Analyses Required			
	Labs	Matrix types	Facilities/ PWSs	IPR- reagent water[2]	IPR- sample matrix [3]	MS/MSD	MDL[4]
Tier 1-Single-lab WW/DW- First matrix type or first PWS	1	1	1	4	4	2[5]	7
WW- Each addt'l matrix type (8 max.) from any industrial category	1	1	1	0[6]	0[6]	2[5]	0[6]
DW- Each addt'l PWS (2 max.)	1	1	1	0[6]	0[6]	2[5]	0[6]
Tier 2-Multi-lab, single matrix type WW/DW- Each matrix type in a single industrial category	3	1	3	12	0	6[7]	21
Tier 3-Multi-lab, multiple matrix types WW only- All matrix types, all industrial categories	9[8]	9	9	36	0	18[7]	63

(1) Numbers of analyses in this table do not include background analyses or additional QC tests such as calibration, blanks, etc. Validation requirements are based on the intended application of the method. Method application would be designated by tier for wastewater (WW) and drinking water (DW) programs. Three would be the maximum number of public water systems (PWSs) that would be required to validate a new or modified drinking water method at Tier 1 or 2. Nine would be the maximum number of matrix types (or facilities) that would be required to validate a new or modified wastewater method at Tier 1 or 3; at Tier 2 the number would be three matrix types.

(2) IPR reagent water analyses would be used to validate a method modification and to establish QC acceptance criteria for initial precision and recovery (IPR) and ongoing precision and recovery (OPR) for a new method. The required number of IPR analyses, except as noted under footnote 7, would be four times the number of laboratories required to validate a method modification or new method because each laboratory would perform a 4-replicate IPR test.

(3) IPR sample matrix analyses would be used to establish QC acceptance criteria for matrix spike/matrix spike duplicate (MS/MSD) recovery and precision for a Tier 1 new method only. Would not be required for validation of Tier 2 or 3 new methods because this variability data would be obtained from MS/MSD tests. Would not be required for validation of a method modification because MS/MSD data from the reference method would be used.

Table 4-2. Summary of Validation Requirements for New Methods and Method Modifications[1] (cont'd)

(4) A method detection limit (MDL) test would be performed in each laboratory using the new or modified method. 40 CFR part 136 Appendix B requires a minimum of seven analyses per laboratory to determine an MDL. Each lab involved in validation of a wastewater modification would demonstrate that the modified method would achieve the detection limits specified in the regulations at 40 CFR parts 136 and 141 and/or in chapter 6 of the Streamlining Guide (EPA 1996a).

(5) MS/MSD analyses would be required only for a method modification because, for new methods, the MS/MSD QC acceptance criteria would be established by the 4-replicate sample matrix IPR test. For modified methods, the MS/MSD test would demonstrate that the reference method MS/MSD QC acceptance criteria have been met.

(6) The MDL, reagent water IPR, and sample matrix IPR tests would not have to be repeated after the first matrix type, facility, or PWS was validated.

(7) For validation of a new method, the MS/MSD analyses would establish QC acceptance criteria for MS/MSD recovery and precision. For validation of a method modification, the MS/MSD analyses would demonstrate that reference method MS/MSD recovery and precision have been met. The required number of MS/MSD analyses would be two times the number of facilities, PWSs or matrix types tested.

(8) The number of laboratories and samples would vary if a conventional interlaboratory study is used.

The tiered approach to validating new and modified methods, presented in Table 4-2, accommodates variability in the analytical performance of a method that can be attributed to the type of sample analyzed. This variability is termed a **matrix effect** and can be observed in samples taken at different locations in matrices of the same type (intramatrix) or in samples from different locations and in different matrix types (intermatrix). Under the streamlining initiative, each successive tier addresses matrix effects to a greater degree through increasing levels of **sample matrix effect validation**, broadly defined as a test of the extent to which differences, if any, in method performance could be attributed to variability between samples obtained from different industrial matrices, facilities, or PWSs. Matrix effects need to be tested by the IPR sample matrix and MS/MSD analyses listed in Table 4-2. Intramatrix effects need to be tested in water samples taken from different PWSs or from different waste streams. Intermatrix effects need to be validated on a group of samples taken from discharge samples collected from several different industrial categories. In all cases, the laboratory must try to determine if the measurement result for the target analyte using a new or modified method differs from the result obtained in a reagent water matrix or in a previously validated matrix type or PWS sample.

As shown in Table 4-2, a **Tier 1** new or modified method is validated in a single laboratory on one or more matrix types obtained from one or more facilities, or on samples obtained from one or more PWSs. Validation of additional facilities or PWSs requires analysis of MS/MSD samples for each additional facility or PWS. However, in response to stakeholder requests that there should be

some maximum number of single-laboratory validations after which further validation would be unnecessary because sample matrix effects would have been sufficiently addressed, EPA has included a provision for a maximum number of matrix type, facility, or PWS analyses for Tier 1 methods. For a wastewater method, the maximum number of matrix types or facilities tested under Tier 1 is nine, each from a different industrial category or subcategory. For a drinking water method, the maximum number of PWS samples tested under Tier 1 is three samples, each from a PWS with different water quality characteristics. Validation in three PWSs, rather than nine, is required because three is consistent with the validation data in many EPA drinking water methods and because the variability in drinking water samples (and therefore the probability of matrix effects) is usually less in drinking water samples than in wastewater samples.

Tier 2 validation is applicable to one or more matrix types within a single industrial category or subcategory. Because Tier 2 new and modified methods apply to each matrix across all laboratories, EPA developed Tier 2 validation requirements to incorporate intramatrix variability. Tier 2 requires validation of the method in drinking water samples obtained from three PWSs, or wastewater samples of one or more matrix types obtained from three or more facilities within a single industrial category or subcategory. Because the drinking water program regulates only one matrix type, drinking (potable) water, Tier 2 results in nationwide approval for a drinking water method.

Tier 3 validation is applicable to the wastewater program and applies to all matrix types in all industrial categories. Consequently, Tier 3 validation requirements include provisions to account for both intramatrix and intermatrix variability. Tier 3 requires validation of the method in wastewater samples of up to nine matrix types obtained from nine different facilities. Tier 3 validation applies to the wastewater program which regulates several industrial categories, each of which may contain more than one matrix type. Tier 3 does not apply to the drinking water program because the drinking water program regulates only one matrix type.

For all multi-matrix tiers, it is extremely important to select suitable samples and matrix types for validation. The matrix types, facilities, or PWSs selected for validation need to have sufficiently different water quality characteristics so that the matrix effects, if any, can be observed. Proposed criteria for selecting matrix types, facilities, or PWSs from which to obtain samples for validation are specified in section 4.4.1.

4.3 Description of Tier 1, 2, and 3 Validation Studies

Ideally, a method modification or a new method should be validated through a classical interlaboratory method validation study of the type used historically by EPA, ASTM, AOAC-International, and other organizations. EPA recognizes, however, that a formal interlaboratory method validation may be prohibitively costly to implement, especially for small laboratories and regulated entities. Therefore, EPA has developed a three-tiered, cost-effective approach to method validation. The tiered approach to validation encourages laboratories to take advantage of new technologies, overcome matrix interference problems, lower detection limits, improve the reliability of results, lower the costs of measurements, and improve overall laboratory productivity without undertaking costly and time-consuming interlaboratory studies.

Tier 1 is expected to be used by commercial laboratories, dischargers, and state and municipal laboratories repetitively testing samples from the same site(s) on a routine basis. **Tier 2** is expected to be used by water supply laboratories, dischargers, and state and municipal laboratories repetitively testing samples from multiple sites within the same industrial category on a routine basis. **Tier 3** is expected to be used by vendors, commercial laboratories, dischargers, and state and municipal laboratories testing a wide variety of sample matrices from diverse sites. Vendors seeking approval of a new technology would also be expected to use Tier 3.

4.3.1 Tier 1 Validation Studies

The primary intent of Tier 1 is to allow use of a new or modified method by a single laboratory. Tier 1 can be applied to a single matrix type or, for drinking water, a single PWS. It also can be applied to multiple matrix types or multiple PWSs.

Tier 1 - Single matrix type/single PWS

Tier 1-Single matrix type/single PWS validation studies are performed in a single laboratory on a single matrix type or on a sample matrix from a single PWS. Results of the validation study and the method modification are applicable in this laboratory to this matrix type or PWS only and cannot be used by another laboratory or for another matrix type or PWS.

Tier 1 - Multiple matrix types

For wastewater, if a laboratory intends to apply the method to more than one matrix type, the laboratory must validate the method on each matrix type, to a limit of nine matrix types. Table 4-2 specifies the specific requirements for the first matrix type and those for each additional matrix type. Some laboratories may be testing multiple matrix types for the same analytes using the same modified method. This raises the question of the number of matrix types to which the modification must be applied to demonstrate that it will likely be successful for all other matrix types. In responding to this question, EPA believes that the number certainly cannot be greater than the number required for validation of a method for nationwide use (nine) and has, therefore, established nine different matrix types as the number after which a test on each subsequent matrix type is not required. The matrices that must be tested for validation of a method for wastewater are given in Table 4-3.

As with a Tier 1-Single matrix type/PWS validation study, Tier 1-Multiple matrix type validation studies are performed in a single laboratory and, therefore, cannot be transferred to another laboratory. If a method is validated by a single laboratory in two to eight discrete matrix types, the validation is applicable to those matrix types only. However, once a laboratory has validated the method on nine matrix types, and those matrix types possess the characteristics required in Table 4-3, the validation is applicable to all other matrix types.

If results of Tier 1-Multiple matrix type validation studies are to be applied to a different medium (e.g., air, water, soil, sludge), each medium must be represented in the samples tested in the validation study.

Table 4-3

Wastewater Matrices Required for Multiple-Matrix Validation Studies

1. Effluent from a publicly owned treatment works (POTW)
2. ASTM D 5905 - 96, *Standard Specification for Substitute Wastewater*
3. Sewage sludge, if sludge will be in the permit
4. ASTM D 1141 - 90 (Reapproved 1992), *Standard Specification for Substitute Ocean Water*, if ocean water will be in the permit
5. Drinking water, if the method will be applied to drinking water samples
6. Untreated and treated wastewaters to a total of nine matrix types

At least one of the above wastewater matrix types must have at least one of the following characteristics:

- Total suspended solids (TSS) greater than 40 mg/L
- Total dissolved solids (TDS) greater than 100 mg/L
- Oil and grease greater than 20 mg/L
- NaCl greater than 120 mg/L
- $CaCO_3$ greater than 140 mg/L

Tier 1 - Multiple PWSs

For drinking water, if a laboratory intends to apply the method to more than one PWS, the laboratory must validate the method on each PWS, to a limit of three PWSs. Table 4-2 specifies the specific validation requirements for the first PWS and those for each additional PWS. EPA proposes to require validation in three rather than nine PWSs, because three is consistent with the validation data in many EPA drinking water methods and because the variability in drinking water samples (and therefore the probability of matrix effects) is usually less in drinking water samples than in wastewater samples.

As with a Tier 1-Single matrix type/PWS validation study, Tier 1 - Multiple PWS validation studies are performed in a single laboratory and, therefore, cannot be transferred to another laboratory. If a method is validated by a single laboratory in one or two PWSs, the validation is applicable to those PWSs only. However, once a laboratory has validated the method in three PWSs and those

PWSs possess different water quality characteristics, as described below, the validation is applicable to all other PWSs.

To test the modified method for potential matrix effects, the three PWS samples must be collected from PWSs with water quality characteristics that are sufficiently different that sample matrix effects, if any, can be observed. In all cases, the laboratory must try to determine if the measurement result for the target analyte using a new or modified method differ from the result obtained in a reagent water matrix or in a previously validated matrix type or PWS sample. Selection of suitable PWSs requires a knowledge of the chemistry of the method. Analysts may review an applicable approved or published method for indications of matrix effects that are unique to the analyte separation and measurement technologies used in the new or modified method. Water quality characteristics that can affect analysis of drinking water samples include, but are not limited to, pH, total organic carbon content, turbidity, total organic halogen content, ionic strength, sulfate contamination, metal contamination, and trihalomethane contamination of the drinking water sample.

4.3.2 Tier 2 Validation Studies

The primary intent of Tier 2 is to allow all regulated entities and laboratories to apply a new or modified method to a single sample matrix type in a single industry. Since drinking water is considered a single matrix type and PWSs represent a single industry, Tier 2 facilitates nationwide use of a new or modified drinking water method.

EPA believes that implementation of Tier 2 will encourage the development and application of techniques that overcome matrix interference problems, lower detection limits, improve the reliability of results, lower the costs of measurements, and improve overall laboratory productivity when analyzing samples from a given industry. For example, the National Council of the American Paper Industry for Air and Stream Improvement, Inc. (NCASI) has suggested a large number of improvements to EPA's proposed and approved methods, with the specific objective of improving method performance in samples from the Pulp, Paper, and Paperboard industrial category. EPA believes that NCASI's suggestions have merit and result in improvements in the reference methods. Through Tier 2, EPA is codifying the ability of NCASI and other industry organizations and associations to improve the approved methods within their respective industries.

Significant industries within Tier 2 are: PWSs, publicly-owned treatment works (POTWs), and individual industrial categories and subcategories that are defined in the regulations at 40 *CFR* parts 405 - 503. At present, there are approximately 42 industrial categories and 650 industrial subcategories defined in the Part 405 - 503 regulations, each of which constitutes an individual industry under the streamlining initiative.

Tier 2 validation studies are performed in a minimum of three laboratories. Samples of the same matrix type (e.g., drinking water, final effluent, extraction-stage effluent,) are collected from a minimum of three separate facilities in the same industrial category or subcategory. A sample from each facility will be sent to each of the laboratories, for a total of nine sample analyses.

For POTWs, if a new or modified method is validated on final effluent only, that method is applicable to final effluent only, and the title of the method must reflect that the method is applicable to final effluent only. If influent to treatment, primary effluent, and sludges will be monitored, the method must be validated separately on these sample matrix types.

In contrast to Tier 1, once a new or modified method has been validated, the validation study results can be transferred to other laboratories, and the other laboratories may freely use the method, as long as the method is applied to analysis of samples of matrix types from within the industrial category or subcategory for which the method has been validated, and as long as the other laboratories meet all of the method's QC acceptance criteria. If the new or modified method is to be applied to another industrial category or subcategory, or to other media or matrix types in the same category or subcategory, the modification must be validated on media/matrix types in each category/subcategory.

4.3.3 Tier 3 Validation Studies

The primary intent of Tier 3 is to allow nationwide use of a new or modified method by all regulated entities and laboratories. The increased flexibility at Tier 3 should allow vendors to establish that new devices and reagents produce results that are acceptable for compliance monitoring purposes, and should allow commercial laboratory chains to apply new technologies or modified techniques throughout their chain of laboratories to a variety of matrices, matrix types, and media.

Tier 3 validation studies are performed in a minimum of nine laboratories, each with a different matrix type at minimum, for a total of nine samples. The minimum requirements for sample matrices that must be used in the validation study are given in Table 4-3. If the method is to be applied to more than one sample medium (e.g., air, water, soil, sludge), a separate validation must be performed on each medium.

When validating a method modification directed at overcoming a matrix interference problem in a specific matrix type, a minimum of three samples representative of those matrix types must be included in the matrix types required by item 6 in Table 4-3. For example, if a modification is intended to overcome matrix interferences associated with effluents containing high concentrations of polymeric materials from indirect industrial discharges in the Thermoplastic Resins subcategory of the Organic Chemicals, Plastics, and Synthetic Fibers industrial category, the modification must be tested on a minimum of three such discharges. Where possible, EPA will assist the purveyor of a method modification in identifying sources for samples of such discharges.

4.4 Development of a Validation Study Plan

Prior to conducting Tier 1, 2, or 3 validation studies, the organization responsible for conducting the study should prepare a detailed study plan. For a simple method modification made at Tier 1, a detailed study plan may be unnecessary if the modification is straightforward and easily understood by the analyst and regulatory authority. In such a case, a simplified study statement may suffice.

The validation study plan should contain the elements described in sections 4.4.1 through 4.4.6.

4.4.1 Background

The Background section of the validation study plan must:

- Identify the method as a new method or a modification of a reference method.
- Include a method summary.
- If a modification, cite the organization and method number (given in 40 CFR parts 136, 141, and 405 - 503) for the reference method.
- If a modification, describe the reasons for and extent of the modification, the logic behind the technical approach to the modification, and the result of the modification.
- If a new method, describe the rationale for developing the method and explain how the method meets the criteria for a new method specified in section 2 of this guide.
- Identify the matrices, matrix types, and/or media to which the method is believed to be applicable.
- List the analytes measured by the method or modification including corresponding CAS Registry or EMMI numbers.
- Indicate whether any, some, or all known metabolites, decomposition products, or known commercial formulations containing the analyte are included in the measurement. (For example, a method designed to measure acid herbicides should include the ability to measure the acids and salts of these analytes.)

4.4.2 Objectives

The Objectives section of the validation study plan should describe overall objectives and data quality objectives of the study.

4.4.3 Study Management

The Study Management section of the validation study plan should:

- Identify the organization responsible for managing the study.
- Identify laboratories, facilities, and other organizations that will participate in the study.
- Delineate the study schedule.

4.4.4 Technical Approach

The Technical Approach section of the validation study plan should:

- Indicate at which Tier level the study will be performed.
- Describe the approach that will be followed by each organization involved in the study.
- Describe how sample matrices and participating laboratories will be selected.
- Explain how samples will be collected and distributed.
- Specify the numbers and types of analyses to be performed by the participating laboratories.
- Describe how analyses are to be performed.

4.4.5 Data Reporting and Evaluation

This section of the validation study plan should explain the procedures that will be followed for reporting and validating study data, and should address statistical analysis of study results.

4.4.6 Limitations

The Limitations section of the validation study plan should explain any limiting factors related to the scope of the study.

4.5 Detailed Procedures for Conducting Tier 1, 2, and 3 Validation Studies

When validating new or modified methods, laboratories must adhere to the standardized QC described in Chapter 3 and detailed in the new or modified method. Laboratories must use a reference matrix (usually, reagent water) and field samples for the validation study.

4.5.1 Optional Preliminary Testing

Although preliminary testing of the new or modified method is not required, many users may wish to conduct such studies prior to performing all of the required tests outlined in Sections 4.6.3-4.6.11 below. Performance of preliminary testing may help organizations identify and correct problems with the method prior to the more extensive and costly method validation study. Typical preliminary performance testing may include a determination of the method detection limit (MDL), analysis of initial precision and recovery (IPR) samples, and ruggedness tests. If such preliminary tests are performed and yield results that suggest further revision of the method is unnecessary, the preliminary test results may be used to fulfill the MDL or IPR test requirements described in Sections 4.6.3 and 4.6.5. If, however, changes are made to the procedures as a result of the preliminary tests, those tests must be repeated as part of the full validation study described below.

4.5.2 Method Compilation

Prior to conducting a complete validation study, the organization responsible for developing or modifying the method should detail the full method in accordance with EPA's *Guidelines and Format for Methods to be Proposed at 40 CFR Parts 136 or 141*. If the organization that develops a new method is a consensus standards organization or government organization with a standardized format, that format may be used. The documented method should be distributed to each laboratory

participating in the validation study to ensure that each laboratory is validating the same set of procedures.

4.5.3 Method Detection Limit Study

Each laboratory participating in the Tier 1, 2, or 3 validation study shall use the procedures specified in the new or modified method and perform an MDL study in accordance with the procedure given at 40 *CFR* part 136, Appendix B.

- If the validation study is of a modified method, each laboratory participating in the study must demonstrate an MDL that meets the criteria specified in the reference method or in Section 6.3.2.9 of this Guide. For wastewater methods, the MDL must be equal to or less than the MDL of the reference method or less than 1/10 the regulatory compliance limit, whichever is greater. This allowance of a higher MDL for a modified wastewater method to support a regulatory compliance limit recognizes that a method modification that overcomes interferences may not achieve as low an MDL as the reference method but is potentially more valuable in allowing determination of the analyte(s) of interest at the regulatory compliance limit in a complex sample matrix.

- If the validation study is of a new wastewater method, the organization responsible for development of the new method must use the results of the MDL study to determine a minimum level (ML) of quantitation as described in Chapter 3. Determination of an ML for new drinking water methods is encouraged but not required, because the regulations at 40 CFR part 141 specify detection and sometimes quantitation limits for all regulated analytes.

Each laboratory must perform its MDL study on an instrument that is calibrated at a range that will encompass the ML.

4.5.4 Calibration

Following completion of the MDL study, each laboratory participating in the study must perform a multi-point calibration in accordance with the procedures specified in the new or modified method. However, a single-point calibration is allowed if the < 2% relative standard deviation (RSD) criteria at Section 3.3.1 of this guide are met.

- If the validation study is of a modified method, each laboratory participating in the study must demonstrate that it can meet the linearity criterion and an ML or other quantitation level that is specified in the reference method or, as may often be the case for drinking water methods, in the applicable regulations.

- If the validation study is of a new method, the organization responsible for development of the method must use the results of the validation study to develop a linearity criterion as described in Chapter 3.

4.5.5 Initial Precision and Recovery

After successfully calibrating the instrument, each laboratory participating in the study shall perform initial precision and recovery (IPR) analyses using the procedures specified in the method to analyze four spiked reagent water replicates.

- If the validation study is of a modified method, each laboratory participating in the study must demonstrate that it can meet the IPR precision and recovery criteria given in the reference method.

- If the validation study is of a new method, the organization responsible for development of the method must use the results of these IPR analyses to develop precision and recovery criteria as described in Chapter 3.

For a new method, the concentration of the IPR samples must be stated in the method. As described in Chapter 3, this concentration should be between one and five times the ML.

4.5.6 Field Sample Analyses

After laboratories participating in the Tier 1, 2, or 3 validation study have successfully completed the IPR analyses, the new method or modification is validated on the matrix type(s) chosen for the validation study. The numbers of analyses required are described below.

4.5.6.1 Tier 1 - Single Matrix Type/Single PWS Validation Studies

In a Tier 1-Single matrix type/PWS study performed to validate a method modification, the laboratory must determine the background concentration of an unspiked sample prior to analyzing an MS/MSD pair for the matrix being tested, for a total of three field sample analyses (background, MS, and MSD). Each laboratory participating in the study must demonstrate that it can meet the MS/MSD precision and recovery criteria given in the reference method.

In a Tier 1 - Single matrix type/PWS study performed to validate a new method, the laboratory must analyze four spiked replicates of the matrix type to which the new or modified method will be applied. The replicate samples must be spiked with the analyte(s) of interest at either the concentration specified in the reference method, at a concentration one to five times the background concentration of the analyte(s) in the sample, or at two to five times the ML, whichever is greater. In other words, the laboratory will perform an IPR test in the matrix type of interest. Prior to spiking the replicate samples, the laboratory must determine the background concentration of an unspiked aliquot. In all, Tier 1-Single matrix type/PWS validation studies of new methods will require analysis of five field samples (one background and four matrix). The organization responsible for developing the method must use the results of these sample analyses to develop MS/MSD precision and recovery criteria as described in Chapter 3.

4.5.6.2 Tier 1 - Multiple Matrix Type Validation Studies

In Tier 1-Multiple matrix type studies performed to validate new or modified methods, the laboratory must determine the background concentration and analyze an MS/MSD pair for each matrix type being tested, up to a total of nine matrix types. Since three field sample analyses are required for each matrix type (one background, one MS, and one MSD), and between two and nine matrix types may be tested, a Tier 1-Multiple matrix type validation study will require analysis of 6 - 27 samples.

- If the validation study is of a modified method, each laboratory participating in the study must demonstrate that it can meet the MS/MSD precision and recovery criteria given in the reference method.

- If the validation study is of a new method, the organization responsible for developing the method must use the results of these sample analyses to develop MS/MSD precision and recovery criteria as described in Chapter 3.

4.5.6.3 Tier 1 - Multiple PWSs

In Tier 1-Multiple PWSs studies performed to validate new or modified methods, the laboratory must determine the background concentration and analyze an MS/MSD pair for each PWS sample being tested, up to a total of three PWS samples. Since three field sample analyses are required for each PWS sample (one background, one MS, and one MSD), and between two and three PWS samples may be tested, a Tier 1-Multiple PWSs validation study will require analysis of 6 - 9 samples.

- If the validation study is of a modified method, each laboratory participating in the study must demonstrate that it can meet the MS/MSD precision and recovery criteria given in the reference method.

- If the validation study is of a new method, the organization responsible for developing the method must use the results of these sample analyses to develop MS/MSD precision and recovery criteria as described in Chapter 3.

4.5.6.4 Tier 2 Validation Studies

In a Tier 2 validation study, each of the three laboratories will determine the background concentration and analyze an MS/MSD pair for each of the three samples received. Because there are three laboratories, each of which performs three analyses (one background, one MS, and one MSD) on each of the three samples received, Tier 2 validation studies will require analysis of 27 samples.

- If the validation study is of a modified method, each laboratory participating in the study must demonstrate that it can meet the MS/MSD precision and recovery criteria given in the reference method.

- If the validation study is of a new method, the organization responsible for developing the method must use the results of these sample analyses to develop MS/MSD precision and recovery criteria as described in Chapter 3.

4.5.6.5 Tier 3 Validation Studies

In a Tier 3 validation study, each of the nine laboratories participating in the study will determine the background concentration and analyze an MS/MSD pair on the sample it receives. Since there are a total of nine laboratories, each performing three field sample analyses (one background, one MS, and one MSD), a Tier 3 validation study will require analysis of 27 samples.

- If the validation study is of a modified method, each laboratory participating in the study must demonstrate that it can meet the MS/MSD precision and recovery criteria given in the reference method.

- If the validation study is of a new method, the organization responsible for developing the method must use the results of these sample analyses to develop MS/MSD precision and recovery criteria as described in Chapter 3.

4.5.7 Ongoing Precision and Recovery

If the field samples discussed in Section 4.6.6 are analyzed as a batch with the IPR samples, analysis of an OPR sample is unnecessary in the validation study. If, however, field samples are analyzed in a different batch or batches, then each laboratory participating in the Tier 1, 2, or 3 validation study must analyze an OPR sample with each batch. The concentration of the OPR sample must be as stated in the method being validated.

- If the validation study is of a modified method, each laboratory participating in the study laboratory that analyzes an OPR sample must demonstrate that it can meet the OPR recovery criteria given in the reference method.

- If the validation study is of a new method, the organization responsible for developing the method must use the results of the IPR tests described above in Section 4.6.5 to develop OPR recovery criteria as described in Chapter 3.

4.5.8 Calibration Verification

If the field samples discussed in Section 4.6.6 are analyzed on the same shift or in the same set of instrumental determinations as the initial calibration sequence, calibration verification is unnecessary. However, if field samples are analyzed on a different shift or in a different instrument batch, each laboratory participating in the Tier 1, 2, or 3 validation study must verify calibration as described in the method.

- If the validation study is of a modified method, each laboratory participating in the study and verifying calibration must demonstrate that it can meet the acceptance criteria given in the reference method for calibration verification.

- If the validation study is of a new method, the organization responsible for developing the method must use the results of the calibration sequence described above in Section 4.6.4 to develop QC acceptance criteria for the calibration verification analyses as described in Chapter 3.

4.5.9 Contamination Level in Blanks

Each laboratory that participates in a Tier 1, 2, or 3 validation study must prepare and analyze at least one method blank with the sample batch during which the matrix samples are prepared and analyzed. The actual number of blank samples analyzed by each laboratory must meet or exceed the frequency specified in the method.

- If the validation study is of a modified method, each laboratory participating in the study must demonstrate that it can meet the QC acceptance criteria for blanks that are specified in the method.

- If the validation study is of a new method, the organization responsible for developing the method must use the results of these sample analyses to develop QC acceptance criteria for allowable blank contamination as described in Chapter 3.

4.5.10 Surrogate or Labeled Compound Recovery

For methods that use surrogates or labeled compounds, each laboratory participating in the Tier 1, 2, or 3 validation study must spike all field and QC samples with the surrogates/labeled compounds at the concentrations specified in the method.

- If the validation study is of a modified method, each laboratory participating in the study must demonstrate that it can meet the surrogate or labeled compound recovery criteria specified in the reference method.

- If the validation study is of a new method, the organization responsible for developing the method must use the results of these sample analyses to develop surrogate or labeled compound recovery QC acceptance criteria as described in Chapter 3.

4.5.11 Absolute and Relative Retention Time

Each laboratory participating in a Tier 1, 2, or 3 validation study of a chromatographic method must determine the absolute and relative retention times of the analytes of interest.

- If the validation study is of a modified method, each laboratory participating in the study must demonstrate that it can meet the absolute and relative retention time criteria that are specified in the reference method.

- If the validation study is of a new method, the organization responsible for developing the method must use the results of these sample analyses to develop absolute and relative retention time criteria as described in Chapter 3.

4.5.12 New Analytes

As described in Chapter 2, EPA proposes to consider the addition of new analytes to approved methods as acceptable performance-based method modifications under the streamlining initiative. Because these method modifications are performance-based, laboratories will be required to demonstrate equivalency in accordance with the requirements summarized above for other Tier 1, 2, and 3 method modifications. In addition, laboratories are required to either develop QC acceptance criteria for the added analyte, transfer QC acceptance criteria from an analyte with similar chemical characteristics, or transfer QC acceptance criteria from another method with the same analyte.

4.5.13 Further Validation Studies for New Methods

After completing the Tier 1, 2, or 3 validation studies of new methods, the organization responsible for developing the method must document the study results in accordance with Section 4.7 below and submit the results and the method to EPA for review and approval, as described in Chapter 5. If, based on its review of the method, EPA concludes that the method is not sufficiently rugged or reliable for its intended use, EPA may require further method development and further testing to define the stability and reliability of the method. The tests and studies that must be performed in this case are dependent upon the analyte(s) and the analytical system, and will be determined on a case-by-case basis as these situations arise.

4.6 Validation Study Report

Laboratories or other organizations responsible for developing a new or modified method at Tier 1, 2, or 3 must document the results of the validation study in a formal validation study report that is organized and contains the elements described in this section. There is one exception to this rule. For Tier 1 method modifications, the completed Checklists (*Checklist for Initial Demonstration of Method Performance*, *Checklist for Continuing Demonstration of Method Performance*, and *Certification Statement*), along with the raw data and example calculations, are considered adequate to document method equivalency; a full validation study report is not necessary.

The information and supporting data required in the validation study report are sufficient to enable EPA to evaluate a new method for adequacy or to support a claim of equivalent performance for a method modification. Some items are required only for a modification; these are clearly identified below. If data are collected by a contract laboratory, the organization responsible for using the method (i.e. permittee, POTW, PWS, or other regulated entity) is responsible for ensuring that all

method-specified requirements are met by the contract laboratory and that the validation study report contains all required data.

Like the validation study plan, the validation study report contains background information and describes the study design. In addition, the validation study report details the process and results of the study, provides an analysis and discussion of the results, and presents study conclusions. If a validation study plan was prepared, it must be appended to and referenced in the validation study report. The validation study report must identify and discuss any deviations from the study plan that were made in implementing the study.

The validation study report must contain the elements described in sections 4.6.1 through 4.6.11.

4.6.1 Background

The Background section of the validation study report must describe the method (new method or method modification) that was validated and identify the organization responsible for developing the method. This section must:

- Identify the method as a new method or a modification of a reference method.
- Include a method summary.
- If a modification, cite the organization and method number (given in 40 CFR parts 136, 141, and 405 - 503) for the reference method.
- If a modification, describe the reasons for and extent of the modification, the logic behind the technical approach to the modification, and the result of the modification.
- If a new method, describe the rationale for developing the method and explain how the method meets the criteria for a new method specified in section 2 of this guide.
- Identify the matrices, matrix types, and/or media to which the method is believed to be applicable.
- List the analytes measured by the method or modification including corresponding CAS Registry or EMMI numbers. (Alternatively, this information may be provided on the data reporting forms in the Supporting Data appendix to the validation study report.)
- Indicate whether any, some, or all known metabolites, decomposition products, or known commercial formulations containing the analyte are included in the measurement. (For example, a method designed to measure acid herbicides should include the ability to measure the acids and salts of these analytes.)

- State the purpose of the study.

4.6.2 Study Design and Objectives

The Study Design and Objectives section of the validation study report must describe the study design, and identify overall objectives and data quality objectives of the study. Any study limitations must be identified. The validation study plan may be appended to the validation study report to provide the description of the study design. If no validation study plan was prepared, the study design must be described in this section (see section 4.4 for required elements of the study design).

4.6.3 Study Implementation

The Study Implementation section of the validation study report must describe the methodology and approach undertaken in the study. This section must:

- Identify the organization that was responsible for managing the study.
- Identify the laboratories, facilities, and other organizations that participated in the study; describe how participating laboratories were selected; and explain the role of each organization involved in the study.
- Indicate at which Tier level the study was performed.
- Delineate the study schedule that was followed.
- Describe how sample matrices were chosen, including a statement of compliance with Tier requirements for matrix type selection.
- Explain how samples were collected and distributed.
- Specify the numbers and types of analyses performed by the participating laboratories.
- Describes how analyses were performed.
- Identify any problems encountered or deviations from the study plan and their resolution/impact on study performance and/or results.

4.6.4 Data Reporting and Validation

This section of the validation study report must describe the procedures that were used to report and validate study data. Although EPA will not establish a standard format for analytical data submission because of the large variety of formats currently in use, EPA strongly recommends the Department of Energy Environmental Management Electronic Data Deliverable Master Specification (DEEMS) because it will expedite processing of the data review. The DEEMS list contains all of the

data elements that laboratories should submit to document method validation. A DEEMS data element dictionary is provided in Appendix D of this guide.

4.6.5 Results

This section of the validation study report presents the study results. Results must be presented on the Checklists *(Checklist for Initial Demonstration of Method Performance*, *Checklist for Continuing Demonstration of Method Performance,* and *Certification Statement)*, or if space does not allow, results may be submitted in a tabular format attached to the Checklists. Raw data and example calculations are required as part of the results and shall be included in an appendix to the validation study report (see section 4.6.10).

The Checklists, instructions for their completion, and an example set of completed Checklists are provided in Appendix E to this guide. For method modifications, the first two Checklists document the technical details required to establish equivalency; the Certification Statement commits the persons involved in the method modification and their management to the statements made in the Checklists and the supporting information provided. The Checklist performance categories, developed with input from EPA's various programs, were designed to apply to as many of these programs as possible. These Checklists apply equally well to screening and field techniques and state-of-the-art laboratory procedures.

The completed Checklists verify that all QC requirements of the method were met. For modified methods, the Checklists verify that the modified method met all QC acceptance criteria of the reference method, for purposes of assessing method equivalency.

4.6.6 Development of QC Acceptance Criteria

For new methods, the validation study report must contain a section that describes the basis for development of QC acceptance criteria for all of the required QC tests. The requirements for developing QC acceptance criteria are detailed in Chapter 3.

4.6.7 Data Analysis/Discussion

This section of the validation study report must provide a statistical analysis and discussion of the study results. For validation of modified methods, the discussion must address any discrepancies between the results and the QC acceptance criteria of the reference method.

4.6.8 Conclusions

The Conclusions section of the validation study report must describe the conclusions drawn from the study based on the data analysis discussion. The Conclusions section must contain a statement(s) regarding achievement of the study objective(s).

4.6.9 Appendix A - The Method

For new methods, the method, prepared in EPA format (i.e., in accordance with EPA's *Guidelines and Format for Methods to be Proposed at 40 CFR Parts 136 or 141*), must be appended to the validation study report. All new methods must contain QC acceptance criteria for all required QC elements (see Chapter 3).

For modified methods, the modified portion of the reference method, prepared in EPA format, must be appended to the validation study report.

4.6.10 Appendix B - Validation Study Plan

If a validation study plan was prepared, it must be appended to the validation study report.

4.6.11 Appendix C - Supporting Data

The validation study report must be accompanied by raw data and example calculations that support the results presented in the report.

4.6.11.1 Raw Data

The Results section of the validation study report must include raw data that will allow an independent reviewer to verify each determination and calculation performed by the laboratory. This verification consists of tracing the instrument output (peak height, area, or other signal intensity) to the final result reported. The raw data are method specific and may include any of the following:

- Sample numbers or other identifiers used by the both the regulated entity and the laboratory
- Sample preparation (extraction/digestion) dates
- Analysis dates and times
- Sequence of analyses or run logs
- Sample volume
- Extract volume prior to each cleanup step
- Extract volume after each cleanup step
- Final extract volume prior to injection
- Digestion volume
- Titration volume
- Percent solids or percent moisture
- Dilution data, differentiating between dilution of a sample and dilution of an extract or digestate
- Instrument(s) and operating conditions
- GC and/or GC/MS operating conditions, including detailed information on
  - Columns used for determination and confirmation (column length and diameter, stationary phase, solid support, film thickness, etc.)
  - Analysis conditions (temperature programs, flow rates, etc.)
  - Detectors (type, operating conditions, etc.)

- Chromatograms, ion current profiles, bar graph spectra, library search results
- Quantitation reports, data system outputs, and other data to link the raw data to the results reported. (Where these data are edited manually, explanations of why manual intervention was necessary must be included)
- Direct instrument readouts; i.e., strip charts, printer tapes, etc., and other data to support the final results
- Laboratory bench sheets and copies of all pertinent logbook pages for all sample preparation and cleanup steps, and for all other parts of the determination

Raw data are required for all samples, calibrations, verifications, blanks, matrix spikes and duplicates, and other QC analyses required by the reference method. Data must be organized so that an analytical chemist can clearly understand how the analyses were performed. The names, titles, addresses, and telephone numbers of the analysts who performed the analyses and of the quality assurance officer who will verify the analyses must be provided. For instruments involving data systems (e.g., GC/MS), raw data on magnetic tape or disk must be made available on request.

4.6.11.2 Example Calculations

The validation study report must provide example calculations that will allow the data reviewer to determine how the laboratory used the raw data to arrive at the final results. Useful examples include both detected compounds and undetected compounds. If the laboratory or the method employs a standardized reporting level for undetected compounds, this should be made clear in the example, as should adjustments for sample volume, dry weight (solids only), etc.

4.7 Reporting Validation Study Results

Only validation study results for new methods are required to be reported to EPA, although entities can request EPA review of method modification validation study results at Tier 2 and 3. Chapter 5 describes procedures for submitting validation study results for EPA review and approval of new methods and Tier 2 and 3 method modifications.

4.7.1 Reporting Validation Study Results for New Methods

Validation study results for all new methods, regardless of tier, must be submitted to EPA for approval. Guidance for submitting validation study results to EPA and a description of the approval process are provided in Chapter 5. The organization responsible for developing the method also must maintain on file complete records of all validation study documentation, including the study plan, validation study report, completed Checklists, and all other information submitted to EPA.

4.7.2 Reporting Validation Study Results for Method Modifications

Validation study results for modified methods, regardless of tier, need not be submitted to EPA for approval. Rather, the organization responsible for developing the method modification must

maintain on file complete records of all validation study documentation, including the study plan, validation study report, completed Checklists, supporting data, and other information required in section 4.6. Laboratories using the modification also should provide a copy of the validation study report with appendixes to all regulated entities whose samples have been analyzed by the modified method.

Regulated entities must retain validation study reports on file and make the files available for review on request by a permitting authority. All records must be available for review by auditors.

Submission of validation study results for Tier 1, 2, and 3 method modifications is not required because EPA does not intend to formally approve such modifications. Tier 1, 2, and 3 modifications are considered to be approved by EPA as long as all validation study and documentation requirements have been met. For entities wishing to seek public recognition that their procedures have been demonstrated to be acceptable for use, EPA proposes to provide an option for submission of Tier 2 and Tier 3 method modificationsfor EPA approval as described in Chapter 5.